Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Theresa Wurzer

Thesis title: The interaction of Streptomyces-like bacteria and model microorganisms in secondary metabolite production, motility and hemolytic activities - Experimental
Supervisor: RNDr. Alica Chroňáková, Ph.D.

Referee: RNDr. Jan Bobek, Ph.D.
Referee`s affiliation: Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	3

* Choose one

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		24
POINTS IN TOTAL (MAX/AWARDED)	48	43

Comments of the reviewer on the student and the thesis:

The submitted thesis studies hemolysis and cu-cultures of different streptomycete strains from a collection. Hundreds of different species were thus analyzed. Additional PCR experiments searched for hemolytic genes known from *S. coelicolor*. The work encompasses a lot of wet experimental analyses that make a promising starting point for a selection of species of possible clinical importance.

In my opinion, on the bachelor thesis level, the amount of work performed is fantastic. This is also the reason of the number of my comments below, which arise purely from my interest in the topic and should not decrease author's credit.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Introduction

1. In the Introduction you nicely describe relationships between different organisms. What type of relationship do you expect in your co-cultivation tests? Would any of closely growing bacteria profit?
2. "Autoinducing peptides (AIPs) are used by Gram-positive bacteria... ... In contrast, Gram-negative bacteria use small molecules as autoinducers: either acyl-homoserine lactones..." Do you know anything about autoinducer molecules in *Streptomyces*?
3. Chapter 3.3 Interaction of streptomycetes with human pathogens- Despite promising title, the chapter does not contain any interaction with streptomycetes published up-to-date.
4. Could author complement examples of metabolites acting synergistically or contingently?
5. Author mentions that "... microbial metabolites are used in different infectious diseases. Additionally, other applications for microbial compounds have been found which include not only human and animal therapy but also agriculture. Moreover, compounds of microbial origin are used for many non-infectious diseases (including for example cancer and heart disease). A very important role is the use in prophylaxis as well as in immunosuppression..." Could you add examples of such useful compounds?
6. p.11 polyene metabolites: "Examples are: (I) filipins, which lead to the formation of large aggregates within the erythrocyte membrane and thus to perforations that are permeable for hemoglobin; (II) amphotericin B and nystatin, which form smaller aggregates within the membranes, permeable for molecules of low weight (Knopik-Skrocka & Bielawski, 2002); (III) candicidin; (IV) pimaricin (Caffrey, et al., 2016)." Please add description and suitable reference

where they are missing. Which of polyene antibiotics are produced by streptomyces?

7. I disagree that "*S. coelicolor* is growing on blood agar and produces a hemolytic zone similar to beta-hemolysis."
8. There are missing references for S-hemolysin-encoding genes SCO1782, SCO2534 and SCO4978.
9. Pseudomonas aeruginosa and Staphylococcus aureus - Why exactly these pathogens were selected for your assay?
10. How did you tested motility (see also q. 26)? Is STAU motile (this was partially commented in the discussion part)?

Material and methods

11. p.15 "...the majority of the clinical strains were isolated from decontaminated sputum (low respiratory tract)." If not from sputum, where do they come from?
12. Table 3
    - There should be "hemolytic activity" instead of "activity"
    - Also "T-type" instead of "t-test" (the same confusion can be found in Discussion, p.40)
    - I disagree that *S. coelicolor* possesses beta-hemolytic activity. In any case, it is a pity that *S. coelicolor* as a control strain was not included in all the analyses.
    - it is not clear what "x" stands for – Does it mean performed test or positive test result? Anyway, when presenting such a comprehensive table, it is a pity that it does not present results (like +/-, or by simplified quantification, like "+++"). This would better demonstrate results, especially of this work, where demonstration of results is somehow unhandy (see also comment 24). I would appreciate if results were included in the Table 3. Then it would be possible to cluster *Streptomyces* species according their properties.
13. Could author somehow comment on the hemolytic activity of STAU and PSAE?
14. p. 19 "All cultivation processes were performed in a laminar box." - Inoculation instead of cultivation
15. p.20 "After 3-4 days, the colonies were observed and the growth and hemolytic activity were recoded." – How was the growth recorded, is it presented somewhere in the thesis?
16. Fig.5 Why is the crossed inoculation called T test? Why were these tests inoculated at different time whereas those parallel were inoculated at once?
17. p.22 "About 100 Streptomyces strains..." Please specify exact number. Nevertheless, this is extremely laborious process to isolate DNA from 100 samples. Why did not you performed just colony pcr - in *Streptomyces* this can be achieved by high temp or in DMSO.
18. p.22 "...centrifuged for 5 minutes at 12000 rpm at room temperature." rpm does not indicate conditions of centrifugation without the rotor specification. Better to specify g-force.
19. p. 23 Please, could you be more specific, how the primers for 16S rDNA PCR were designed? Which template was used as a positive ctrl?
20. "The electrophoresis was run at 110 V and 265 mV." What does 265mV mean? Mind that the current (in mA) would not be constant.
21. How did you work with eth br.? What safety rules are important? Could you describe "ethidium bromide bath" and how did you handled with it? Nowadays, there are more safe stains that can be put directly into the gel.
22. p24 Please, explain, why you have used strain TR42 instead of *S. coelicolor* as a reference strain for primer design – is this strain more related to the collected streptomycetes? I would also appreciate more detailed up-to-date description of the TR42.

Results

23. Why is the hemolytic activity assay in the co-cultivation chapter?
24. In my opinion categorization of hemolytic activity and subsequent statistical analyses based on it presented in graphs are cumbersome and hardly understandable for readers. Instead of this categorization I would suggest clustering the streptomycete strains based on their hemolytic change. Then, based on this, author could present clearer and more elucidating charts to overview streptomyces properties.
25. What are the double zones mentioned in Table 10? Do you have any explanation, how could they develop?
26. You analyzed width of inoculated cell lines. How can you assure the same width and bacterial mass of inoculation (when using wire loop)? Is this the method how you tested motility mentioned in discussion?
27. How was the sporulation of *Streptomyces* categorized??
28. You presented a lot of data. Please, could you comment on what could they be good for and where is their potential for future projects?

Conclusion:

In conclusion, I

recommend / ~~do not recommend*~~

the thesis for the defense and I suggest the grade 1 .[2]

In **Prague** date 12. 9. 2019

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signature

[2] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).